METHODS OF USING BISPECIFIC ANTIGEN-BINDING CONSTRUCTS TARGETING HER2

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 15/526,888, pending, which is a 371 US national phase application of International application no. PCT/CA2015/051238, filed on Nov. 26, 2015, and claims priority to U.S. provisional application No. 62/166,844, filed May 27, 2015; each of which is herein incorporated by reference, in its entirety, for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which will be submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Sep. 25, 2020, is named ZWI-039WOUSC1_sequence-listing.txt, and is 275,091 bytes in size.

BACKGROUND

[0003] The majority of current marketed antibody therapeutics are bivalent monospecific antibodies optimized and selected for high affinity binding and avidity conferred by the two antigen-binding domains. Afucosylation or enhancement of FcgR binding by mutagenesis have been employed to render antibodies more efficacious via antibody Fc dependent cell cytotoxicity mechanisms. Afucyosylated antibodies or antibodies with enhanced FcgR binding still suffer from incomplete therapeutic efficacy in clinical testing and marketed drug status has yet to be achieved for any of these antibodies. Typical bivalent antibodies conjugated to toxins (antibody drug conjugates) are more efficacious but broader clinical utility is limited by dose-limiting toxicity.

[0004] Therapeutic antibodies would ideally possess certain minimal characteristics, including target specificity, biostability, bioavailability and biodistribution following administration to a subject patient, and sufficient target binding affinity and high target occupancy to maximize antibody dependent therapeutic effects. Typically therapeutic antibodies are monospecific. Monospecific targeting however does not address other target epitopes that may be relevant in signaling and disease pathogenesis, allowing for drug resistance and escape mechanism. Some of the current therapeutic paradigms call for the use of combination of two therapeutic monospecific antibodies targeting two different epitopes of the same target antigen. One example is the use of a combination of Trastuzumab and Pertuzumab, both targeting the HER2 receptor protein on the surface of some cancer cells, but patients still progress with disease while others with lower HER2 receptor levels (HER2<3+ by Hercept test) show no therapeutic benefit. Therapeutic antibodies targeting HER2 are disclosed in WO 2012/143523 to GenMab and WO 2009/154651 to Genentech. Antibodies are also described in WO 2009/068625 and WO 2009/068631.

[0005] Co-owned patent application number PCT/CA2014/051140 describes HER2 antibodies. Co-owned patent application number PCT/US2014/037401 (WO 2014/182970) describes HER2 antibodies. Co-owned patent application number PCT/CA2013/050358 (WO 2013/166604) describes single arm monovalent antibodies. Co-owned patent applications PCT/CA2011/001238, filed Nov. 4, 2011, PCT/CA2012/050780, filed Nov. 2, 2012, PCT/CA2013/00471, filed May 10, 2013, and PCT/CA2013/050358, filed May 8, 2013 describe therapeutic antibodies. Each is hereby incorporated by reference in their entirety for all purposes.

SUMMARY

[0006] Described herein are methods of using one or more antigen-binding constructs to treat tumors in a subject, e.g., such as gastric, pancreatic, breast, lung, or head and neck tumors. The one or more antigen-binding constructs can comprise a first antigen-binding polypeptide construct which monovalently and specifically binds a HER2 (human epidermal growth factor receptor 2) ECD2 (extracellular domain 2) antigen on a HER2-expressing cell and a second antigen-binding polypeptide construct which monovalently and specifically binds a HER2 ECD4 (extracellular domain 4) antigen on a HER2-expressing cell, first and second linker polypeptides, wherein the first linker polypeptide is operably linked to the first antigen-binding polypeptide construct, and the second linker polypeptide is operably linked to the second antigen-binding polypeptide construct; wherein the linker polypeptides are capable of forming a covalent linkage with each other, wherein at least one of the ECD2- or the ECD4-binding polypeptide constructs is an scFv. In certain embodiments, the ECD2-binding polypeptide construct is an scFv, and the ECD2-binding polypeptide construct is a Fab. In certain embodiments, the ECD2-binding polypeptide construct is a Fab and the ECD4 binding polypeptide construct is an scFv. In some embodiments, both the ECD2- and ECD4-binding polypeptide constructs are scFvs. In some embodiments, the antigen-binding constructs have a dimeric Fc comprising a CH3 sequence. In some embodiments, the Fc is a heterodimer having one or more modifications in the CH3 sequence that promote the formation of a heterodimer with stability comparable to a wild-type homodimeric Fc. In some embodiments, the heterodimeric CH3 sequence has a melting temperature (Tm) of 68° C. or higher.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. **1**A depicts the structure of a biparatopic antibody in a Fab-Fab format. FIGS. **1**B to **1**E depict the structure of possible versions of a biparatopic antibody in an scFv-Fab format. In FIG. **1**B, antigen-binding domain 1 is an scFv, fused to Chain A, while antigen-binding domain 2 is a Fab, fused to Chain B. In FIG. **1**C, antigen-binding domain 1 is a Fab, fused to Chain A, while antigen-binding domain 2 is an scFv, fused to Chain B. In FIG. **1**D, antigen-binding domain 2 is a Fab, fused to Chain A, while antigen-binding domain 1 is an scFv, fused to Chain B. In FIG. **1**E, antigen-binding domain 2 is an scFv, fused to Chain A, while antigen-binding domain 1 is a Fab, fused to Chain B. In FIG. **1**F, both antigen-binding domains are scFvs.

[0008] FIGS. **2**A-**2**C depict the characterization of expression and purification of exemplary anti-HER2 biparatopic antibodies. FIG. **2**A and FIG. **2**B depict the SEC chromatograph of the protein A purified antibody, and non-reducing SDS-PAGE analysis of 10 L expression and purification of v5019. FIG. **2**C depicts the SDS-PAGE analysis of a 25 L expression and purification of v10000.